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Impact of Aeration on the Removal of Organic Matter and Nitrogen Compounds in Constructed Wetlands Treating the Liquid Fraction of Piggery Manure

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Abstract: The increasing demand for sustainable, robust and cost-efficient wastewater treatment techniques strengthen the implementation of constructed wetlands (CWs) in the agricultural sector. In countries like Belgium (Flanders), the compliance of strict water quality standards and surface area requirements have hindered considerably their application. New wetland designs such as aerated CWs, could help to overcome these challenges. This study evaluated the capacity of artificially aerated mesocosm systems to decrease chemical oxygen demand (COD) concentrations below the 125 mgO_2/L limit imposed on installations treating animal manure. The treatment of this high-strength wastewater has been slightly studied via aerated CWs. A three-stage experiment investigated the effect of constant, intermittent and non-aeration regimes on: ammonium volatilisation, the evolution of organic and nitrogen compounds concentrations, and denitrification. The results were assessed through a mixed modelling procedure using SAS 9.4 software. A COD removal between 65% and 58% in constantly and intermittent aerated systems, versus 27% COD removal in the non-aerated system indicated the effectiveness of aeration. However, a dissimilarity was encountered in the removal of nitrogen compounds, resulting in an 82% decrease of nitrate concentrations in the non-aerated system, versus 0.5% and 11% in the aerated ones. Based on the results, this experimental set-up adjusted to field operational conditions can prove that aerated CWs can treat the liquid fraction of piggery manure.

Keywords: Aerated constructed wetlands; chemical oxygen demand; nitrification-denitrification; piggery manure; ammonium

1. Introduction

In Northwest Europe (NWE), several projects regarding waste management and bio-based resource recovery processes have been interlinked and developed based on circular economy principles, in opposition to the traditional linear economy. The aims have been to minimise natural resource needs, the emissions of waste, energy leakage, and promote their practice elsewhere. Flanders in Belgium, being part of the NWE area, is classified as a nitrate vulnerable zone (NVZ), where nutrients resulting from the excess of animal manure should be reduced. The surplus of farm wastes has largely affected the soil, ground and surface water of coastlines and watercourses [1]. Nitrate vulnerable zones are restricted on the use of fertilisers and are obliged to reduce nitrate concentrations in water below the 50 $mgNO_3^-/L$. Since the 2000s, manure management activities involve the capture, storage, spread on the field, treatment, and its re-use as a source (e.g., as biomass for energy production, growth

medium for algal cultivation). Land spreading can be a viable option where there are large areas of available farmland. Alternatively, pig slurry should be treated either for further use (e.g., fertiliser) or proper disposal. One of the available manure management practices consists of a three-stage process. In the primary treatment, pig slurry is separated into a thin/liquid fraction and a thick/solid fraction [2,3]. In secondary treatment, the solid fraction is converted into an exportable product, such as soil enhancers and fertilisers [3]. The liquid fraction can be treated by biological reactors where nitrogen and phosphorus concentrations are depleted, hence, the separation of pig manure into liquid and solid fractions as the primary treatment. The activated sludge treatment and anaerobic digestion (AD) as the secondary treatment are considered the most common and among the best options available amongst manure management techniques [2,4]. Given the high concentrations of nutrients in soil and strict implemented regulations, additional treatments are needed for both fractions of manure [5,6]. One of them is the use of constructed wetlands (CWs) as the tertiary treatment of the liquid fraction of piggery manure. The liquid fraction coming from activated sludge reactors and the liquid digestate from the AD process, can be treated by CWs to reduce the concentrations of remaining nutrients and other pollutants [4,7]. In Flanders, CWs treating wastewater of animal manure can discharge effluents with a maximum of 15 mg/L of total nitrogen (TN), 125 mgO_2/L of chemical oxygen demand (COD), and pH between 6.5 to 8.5 units [8]. There are no specified values regarding the microbial parameters. However, to meet the COD discharge limit, CWs sometimes must be over-dimensioned depending on the mass loadings. Consequently, in regions with limited land availability (e.g., Flanders) the implementation of CWs becomes restricted. As described by Donoso et al.[6], regardless of the size a CW, its discharge must meet the standards limits set by the EU Water Framework Directive. These limits were defined on basis of CWs larger than 50 ha and large waterbodies, but not to actual conditions. Therefore, there is a need to investigate the effect of emerging technologies that are adaptable to the treatment process and allow higher COD removal efficiencies to avoid the over dimensioning of CWs, and help meet the standards.

In this regard, this study evaluates the effect of implementing artificial aeration in CWs to reduce COD concentrations. It is hypothesised that aerated systems favour the depletion of slowly biodegradable or non-biodegradable organic compounds and facilitate meeting discharge limits. However, considering that constant or intermittent aeration could also favour ammonium (NH_4^+) volatilisation and would therefore hinder the denitrification process, the evolution of total nitrogen (TN) and nitrate (NO_3^-) concentrations are also studied.

The different international standardised methods for COD determination do not differentiate between biodegradable and recalcitrant COD fractions [9]. The biological and chemical oxygen demand ratios (BOD/COD), at times used to indicate the biodegradation capacity of organic matter to be broken down, are generally low in influents and effluents of CWs treating the liquid fraction of animal manure [10]. The high influent COD concentrations of approximately 800–1700 mgO_2/L and 50–100 mgO_2/L of BOD (1:17 BOD/COD ratio) after the biological treatment of the liquid fraction of animal manure indicate the low biodegradability of the remaining COD [10]. The constructed wetlands treating the liquid fraction of piggery manure together with plant litter generate fractions of recalcitrant or non-biodegradable organic matter which increase COD concentrations [11–13].

Studies have indicated that CWs have reached BOD and COD removal efficiencies above 80% [2,10,14,15] in Flanders. Several researchers have shown that aerated CWs can potentially enhance the removal of organics and nutrients [4,16–19]. Mostly synthetic, urban or landfill leachate wastewaters have been considered for testing. S. Wu et al. [19] assessed the effect of aeration and effluent recirculation on: (i) organic matter, and (ii) total nitrogen removal of pig manure liquid digestate generated by anaerobic digestion. In this study, the liquid fraction of piggery manure coming from the biological treatment and discharged into a CW was used for analysis. However, the present experimental set-up only considered the effect of aeration and no recirculation of the wastewater. Water was collected and kept in containers where different aeration regimes were applied. The total nitrogen (TN), ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), COD and BOD

concentrations were determined to assess the effect of aeration over time. During the experimental process, three aspects were tested: (i) ammonium volatilisation due to aeration; (ii) the evolution of physicochemical parameters of the wastewater (COD, BOD and nitrogen compounds concentrations); iii) the enhancement of the denitrification process by the addition of an external carbon source. The results were treated through a mixed model procedure and discussed, based on the removal efficiency of the different aeration rates.

2. Materials and Methods

2.1. Experimental Setup

The experiments were performed in four parallel 1 m^3 cube-shaped intermediate bulk containers (named IBC A, B, C, and D) made of white translucent polyethylene and held within a tubular iron cage. The IBCs were kept open with an opened surface of 1m^2 and placed inside a barn located next to a CW treating the liquid fraction of piggery manure. The aeration pipes were installed at the bottom of three of the four IBCs for air supply. Aeration was provided with a membrane blower, type BibusSecoh JDK20, one for each aerated IBC.

Each of the IBCs was filled up to ninety percent of their capacity with normal round expanded clay aggregates (Argex®). Their particle size was between 8 to 16 mm with a dry-weight density of 340 kg/m^3. An approximate volume of 400 L was filled with influent of the CW which was not renewed throughout the whole experiment. The different aeration rates were provided in each of the three IBCs and one was kept without aeration for comparison. When air was provided, the flux was 25 L/min. The aeration cycles were set in a way that IBC A was constantly aerated, whereas IBC B and C represented two intermittent aeration systems with two different aerated/non-aerated cycles every day. For IBC B, the intermittent aeration regime consisted of four hours with aeration followed by four hours without aeration. For IBC C, the intermittent aeration regime consisted of six hours with aeration followed by four hours without aeration. The IBC D served as a not-aerated control. The whole testing period had a duration of four months (June to October 2017).

A schematic representation of the experimental setup is presented in Figure 1.

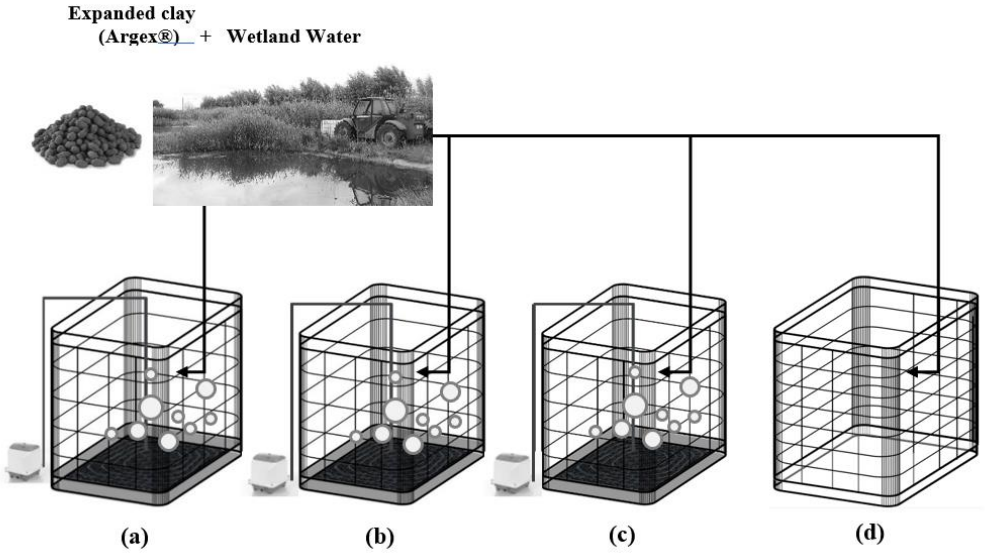

Figure 1. Schematic representation of the operating batch mode intermediate bulk containers (IBCs) used to test the effect on inorganic (TN, NO_3^-, NH_4^+) and organic (chemical oxygen demand (COD), biological oxygen demand (BOD)) chemical concentrations subjected to different aeration rates: (**a**) constant aeration, (**b**) 4 h period with aeration followed by 4 h without aeration, (**c**) 6 h period with aeration followed by 4 h without aeration, (**d**) absence of artificial aeration. Approximately 90% of each IBC capacity was filled with expanded clay and 400 L of influent wetland water.

Three research questions were formulated considering the different aspects to assess during the experimental process. At the beginning or first experimental stage, it was hypothesised that ammonium volatilisation due to active aeration may be substantial. To test this effect, an additional solution of ammonium 15% was added to each IBC to reach an initial concentration of 200 mgNH_4^+-N/L. TN, NO_3^- and NH_4^+ concentrations were determined from the samples collected at the top right and left corners, and from the centre of each container between 40 to 50 cm depth. The daily samples were collected during five consecutive days.

Following this sequence, a six-week stabilisation period of the IBC systems was considered as appropriate to allow biofilm growth prior to proceeding with the second stage of the experiment. The constant and intermittent aeration regimes were kept during this six-week period. While testing a comparable experimental set-up, Gupta et al. [20] and Wu et al. [4] considered a period of two months for the establishment and acclimatisation for microbial communities to populate the substrate particles. In the second stage of the experiment, the effect of aeration was evaluated considering the evolution of COD, BOD, TN, NO_3-N and NH_4^+-N concentrations. The samples were collected at the same spots of each container on a regular basis twice a week during a period of two months.

The third stage of the experiment considered the addition of an external carbon source (glucose) solution in each container. Its influence was tested on the denitrification process which was possibly affected by the continuous and intermittent aeration regimes. Prior the addition of glucose solutions, actual COD and TN concentrations were determined. Based on these concentrations, glucose solutions with a 3:1 COD/N ratio were prepared and added in each container. Then, NO_3^--N and TN concentrations were determined from the samples collected on a daily basis from each IBC during a five-day period.

2.2. Physicochemical Analyses

At every sampling day, regardless of the testing periods, water samples were collected in triplicate from each IBC and preserved at 4 °C. The collected samples were analysed with a maximum holding time of one to two days. This is in agreement with the EPA-600/4-79-20 methods for chemical analysis of water and wastes [21]. The determination of pH, electrical conductivity (EC) and dissolved oxygen (DO) measurements were performed in the laboratory. pH was measured using an 826-pH mobile meter. EC was determined using a four-electrode conductivity sensor model WTW Cond 315i. DO was determined using a WTW Oximeter, model 330i including a CellOx 325 sensor (probe). BOD concentrations were determined through a respirometric method according to the Standard Methods for the Examination of Water and Wastewater [22]. The initial DO concentrations and after 5 days-incubation period at 20 °C ± 1 °C were measured in the BOD bottles. The COD concentrations were determined through spectrophotometric testing using NANOCOLOR® test kits, range 15–160 mg/L COD [23]. The TN concentrations were determined photometrically. First, a Spectroquant® reagent (Crack Set 20) was used to transform organic and inorganic to nitrate. Later, the TN concentrations were determined via test kits with a measuring range of 0.2–20 mg/L NO_3^--N [24]. Likewise, this test kit was used to determine NO_3^- concentrations, with the difference that the initial destruction step was not needed. NH_4^+-N was determined using a Spectroquant® test kit with a measuring range of 0.05–3 mg/L. After the seventh sampling instance, NO_3^- and NH_4^+ were determined by ion chromatography technique using a Methrom 761 Compact Ion Chromatograph.

2.3. Hypotheses Testing Through a Mixed Model Procedure

The removal percentage of each measured variable was estimated in every stage of the experiment. Subsequently, to identify the most efficient aeration frequency in terms of removal, a mixed modelling procedure (PROC MIXED) in SAS 9.4 software was used. Through the development of mixed linear models, the correlation between measurements of the water samples taken at each time point for each IBC system were recorded. The selected statistical analysis was based on its flexibility to model the repeated measurements from the same experimental unit. For the model development, each of the

containers (labelled: "subject"), the different aeration frequencies (labelled: "treatment"), and sampling days (labelled: "time") were considered as categorical variables. pH, EC, DO, COD, TN, NO_3^- and NH_4^+ represented the response variables each in turn. Subject was modelled as a random effect while treatment, time and the interaction between treatment and time represented the fixed effects. The degrees of freedom were estimated using the Satterthwaite approximation [25].

Given the fact that the dependent variables in the model each represented a measurement on the same experimental IBC, the "repeated" statement was used to model the variability within treatment over time. A significance level was set at $p \leq 0.05$. Finally, the compound symmetry (CS) variance covariance structure, which assumes that the correlation between any two measurements from the same treatment is the same, was used. Then, to compare the expected least squares means response between the different treatments, the "lsmeans" statement was used together with the Tukey adjustment method which corrects the p-values for multiple testing. In a general context, the statistically significant estimated effects represent the matching days when the concentrations of treatment (e.g., A and B) significantly change. By assessing the size and the sign (+ or −) of the effects, together with the repeated influence of a specific aeration regime in the concentrations of each variable, overall judgements could be done. Finally, to validate the model estimations and overall findings, the model residuals were plotted against the corresponding fitted values to check for normality.

3. Results

3.1. First Stage: Testing Ammonium Volatilisation

The initial mean concentrations between 165 to 173 mgNH_4^+-N/L in comparison to the mean concentrations determined on the fifth day of the test between 165 to 168 mgNH_4^+-/L, indicate that no significant decrease ($p > 0.05$ based on Wilcoxon rank sum test) was evident in the ammonium concentrations. The ammonium concentrations recorded for treatment A showed more variability than the other treatments, most probably due to the constant aeration in the system. However, the pH values of 6.8–7.5 indicate that only approximately 1% of NH_3 is present, thus no significant NH_4^+ removal could be expected according to pKa (NH_4^+/NH_3) = 9.25 at 25 °C.

Figures 2 and 3 show the mean ammonium concentrations and pH values determined at each IBC during the five-day period of this stage.

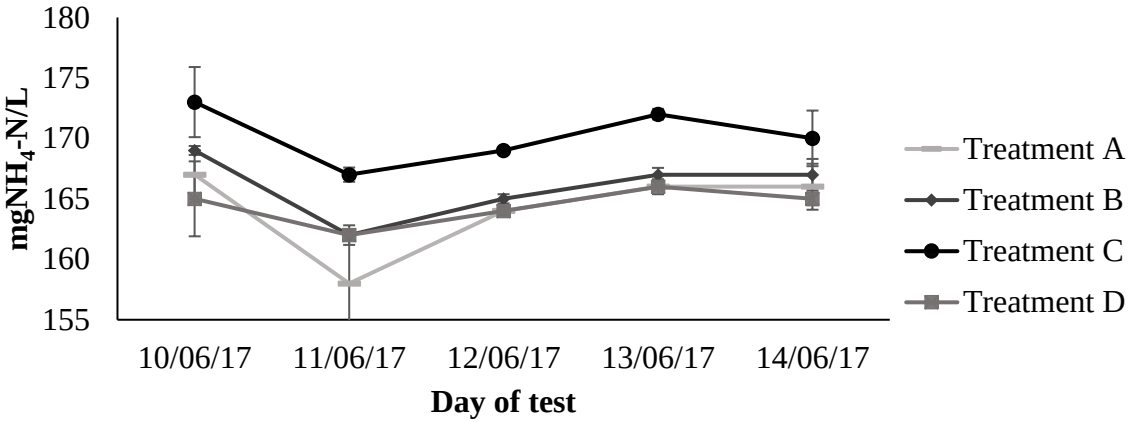

Figure 2. The mean of NH_4^+-N concentrations (mgNH_4^+-N/L) recorded during the ammonium volatilisation test. Treatment A (constant aeration), Treatment B (intermittent aeration 4h AA:4h no-AA), Treatment C (intermittent aeration 6h AA:4h no-AA), Treatment D (no aeration), AA = aeration.

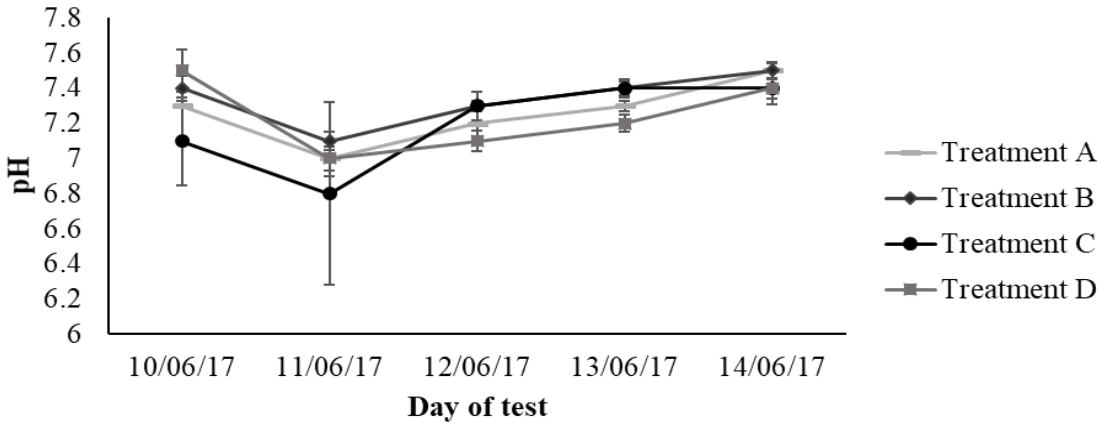

Figure 3. The mean pH values recorded during the ammonium volatilisation test. Treatment A (constant aeration), Treatment B (intermittent aeration 4h AA:4h no-AA), Treatment C (intermittent aeration 6h AA:4h no-AA), Treatment D (no aeration), AA = aeration.

3.2. Second Stage: Evolution of Physicochemical Parameters, Organic and Nitrogen Compound Concentrations under Different Aeration Regimes

The initial and final value of each physicochemical parameter, organic and nitrogen compound concentrations are presented in Appendix A Table A1. Based on these records, the removal percentages of organic and nitrogen compounds were estimated considering the different aeration regimes. The evolution of recorded values was assessed based on Figures 4 and 5. Their statistical relevance was evaluated through the estimates derived from the mixed model procedure. For a better graphical interpretation of the results, Figure 4 shows the evolution of pH, EC, DO, BOD, and COD, whereas, Figure 5 indicates the evolution of nitrogen compounds (NH_4^+-N, NO_3^--N and TN) set against the sampling date.

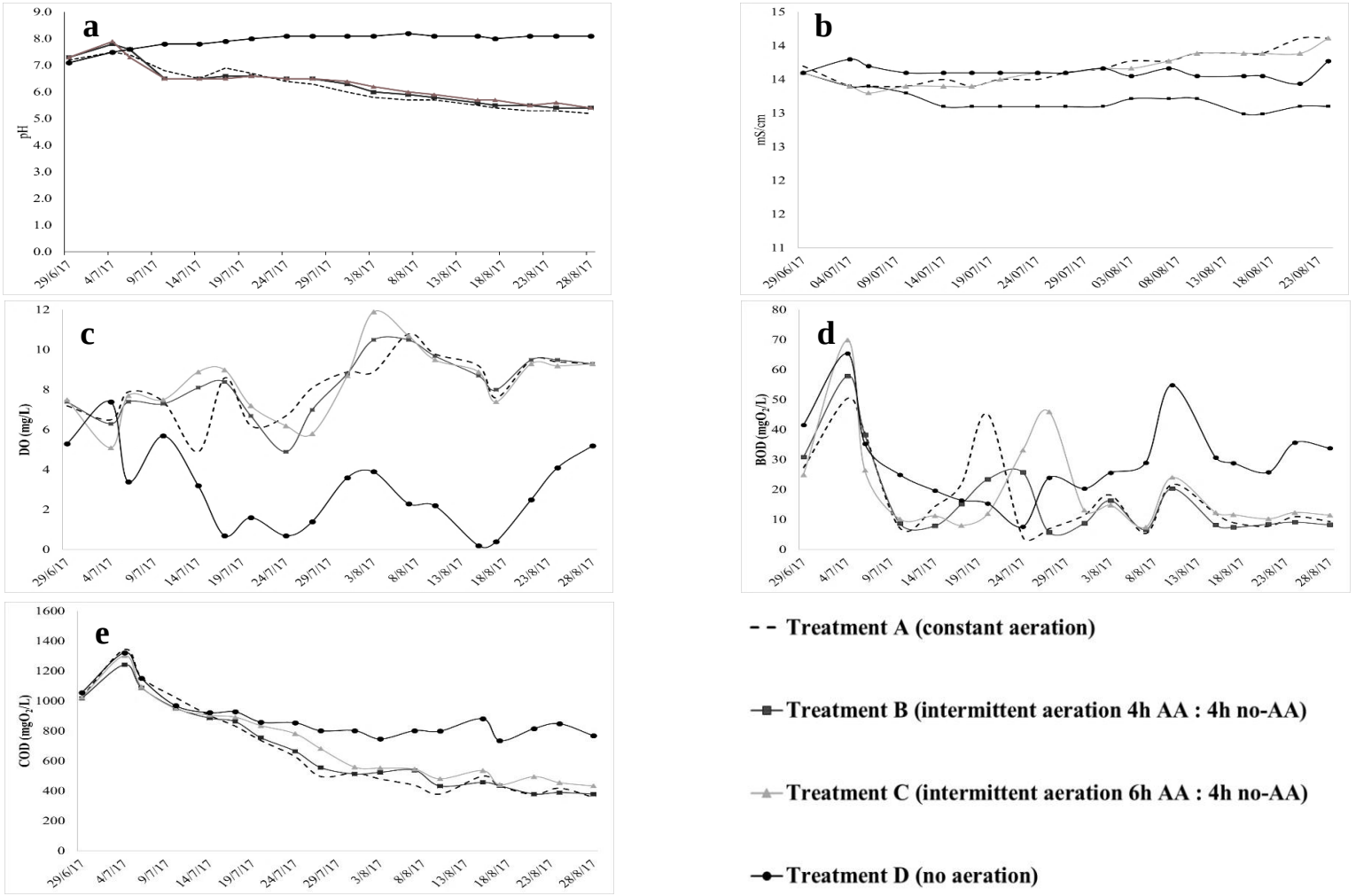

Figure 4. Evolution of (**a**) pH, (**b**) conductivity, (**c**) dissolved oxygen, (**d**) biological oxygen demand, (**e**) chemical oxygen demand in each IBC exposed to different aeration periods. Treatment A (constant aeration), Treatment B (intermittent aeration 4h AA:4h no-AA), Treatment C (intermittent aeration 6h AA:4h no-AA), Treatment D (no aeration), AA = aeration.

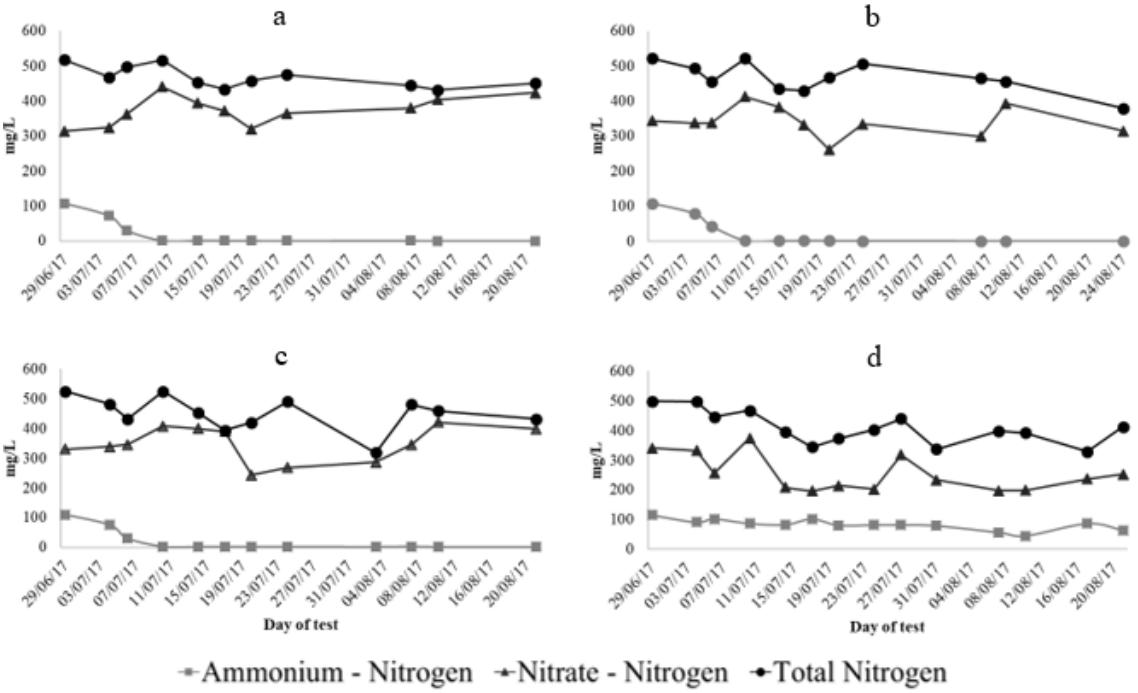

Figure 5. Evolution of ammonium-nitrogen, nitrate-nitrogen and total nitrogen in each IBC exposed to different aeration periods. (**a**) Treatment A (constant aeration), (**b**) Treatment B (intermittent aeration 4h AA:4h no-AA), (**c**) Treatment C (intermittent aeration 6h AA:4h no-AA), (**d**) Treatment D (no aeration), AA = aeration.

The evolution of the three different nitrogen-compounds per treatment was compared in single graphs due to the link in their removal processes. Figure 4a indicates that pH values decreased over time in the IBCs where constant and intermittent aeration was provided. Relatively similar pH values were registered in treatment A (from 7.2 to 5.2), treatment B (from 7.3 to 5.4) and treatment C (from 7.3 to 5.4) after 60 days. An opposite effect was identified in the IBC without aeration where pH increased from 7.1 to 8.1 in the same period. The initial conductivity values 13.6 mS/cm shown in Figure 4b remained relatively stable throughout the whole testing period. On the last testing day of the two-month period, the water samples representing treatment B (13.2 mS/cm), followed by the ones of treatment D (13.6 mS/cm) showed a slight decrease in comparison with the water samples from treatment A and C (14.1 and 14 mS/cm respectively). The DO concentrations (Figure 4c) in treatments A, B, and C increased over time. Again, relatively similar values were recorded among these treatments. Treatment A (from 7.2 to 9.3 mg/L), treatment B (from 7.4 to 9.3 mg/L), treatment C (from 7.5 to 9.3 mg/L) clearly show the effect of aeration provided to each IBC. However, the DO concentrations in treatment D lowered and stayed below the initial 5 mg/L since no air was supplied to the system. It is relevant to state that reported DO concentrations should be considered as an indication that the systems were aerated through the experimental period. However, for quantification purposes and the control of aerated and non-aerated periods, daily and in-situ measurements are recommended.

Figure 4e shows that the COD concentrations decreased gradually over time, but only 27% removal was achieved in the case of treatment D. A removal of 65% was obtained when constant aeration was provided. The concentrations reported from treatment B and C showed that shorter aeration periods resulted in lower removal efficiency (63% and 58% respectively). For the specific case of BOD in Figure 4d, the applied model procedure was not able to estimate the statically significant effects of aeration. Due to fluctuation in the measurements, there was insufficient data to estimate the particular covariance structure [26]. Therefore, the effect of aeration in BOD is considered based on observations and interpreted with caution. The BOD measurements show high variability within the treatments. Only after the 10th sampling day (one-month period), the BOD concentrations in treatments A, B,

and C followed a similar pattern and stabilised at approximately 8 mgO_2/L. In treatment D, the BOD concentrations remained higher at approximately 33 mgO_2/L.

Similar to BOD, NO_3^--N (Figure 5 ▲) measurements showed great variability. In the case of NO_3^--N, the variation amongst the measurements could be attributed to the different analytical methods used. The test kits were used to determine TN, whereas, NO_3^--N and NH_4^+-N were determined by ion chromatography. The high dilution factors were needed to analyse the samples by ion chromatography due to high sensitivity and low detection limits of the equipment. Considering that this fact could have increased the error or variation in the analysis, the results were corroborated and some samples re-analysed using quick test kits. The inconsistent NO_3^--N values evidenced through the comparison with the TN concentration, and after testing similar methodologies, were considered as outliers and removed from the dataset. The overall tendency indicates that NO_3^--N concentrations decreased only in treatment D from 341 mg/L to 223 mg/L, indicating a removal of 35%. The estimated effects of this treatment supported that nitrate concentrations significantly decreased ($p < 0.05$) in the non-aerated container. The ammonium concentrations started to decrease significantly after a one-week period of the second stage of the experiment (Figure 5 ■). A full NH_4^+-N removal was obtained in treatment A, B and C, whereas, a lower removal efficiency of 35% was achieved in treatment D. Looking at Figure 5 ●, it can be stated that there is no clear distinction among the effects of aeration on TN concentrations between treatments. However, the 55% of TN removal achieved by treatment D was just slightly above the 50% removal at treatment A. By assessing the whole testing period through the model procedure estimate effects, the results corroborated that statistically significant effects on the reduction of TN concentrations were mostly recorded in the non-aerated container, and a few other occasions when intermittent aeration, as in treatment C, was applied.

In Appendix B, Table A2 presents the mean values of the physicochemical, organic and nitrogen compounds recorded during the second stage of the experiment. Figure A1a–d represent the diagnostic plots of the mixed modelling procedure for COD, NH_4^+-N, NO_3^--N and TN, respectively. The plots indicated residuals are normally distributed.

3.3. Third Stage: Intensification of Nitrification and Denitrification Processes by the Addition of a Supplementary Carbon Source

Considering the high nitrate concentrations remaining in the systems at the end of the second stage, it was presumed that the denitrification process did not occur completely due to the lack of available organic carbon. Thus, it was expected that through the addition of a supplementary carbon source, the denitrification process could be promoted and consequently nitrate would be removed.

The results presented in Figure 6 showed that the aim of this stage was not achieved. Although nitrate concentrations fluctuated throughout the testing period, these remained high and no significant nitrate removal was obtained. In treatments A, B and C, nitrate removal was only 6%, 0.5% and 11%, respectively. Conversely, 82% of nitrate was significantly removed in treatment D ($p > 0.05$ based on Wilcoxon rank sum test). Figure 6 presents the mean concentrations of NO_3^-N in Figure 6a, TN in Figure 6b and COD in Figure 6c, prior (marked as **) and after the addition on the glucose solution.

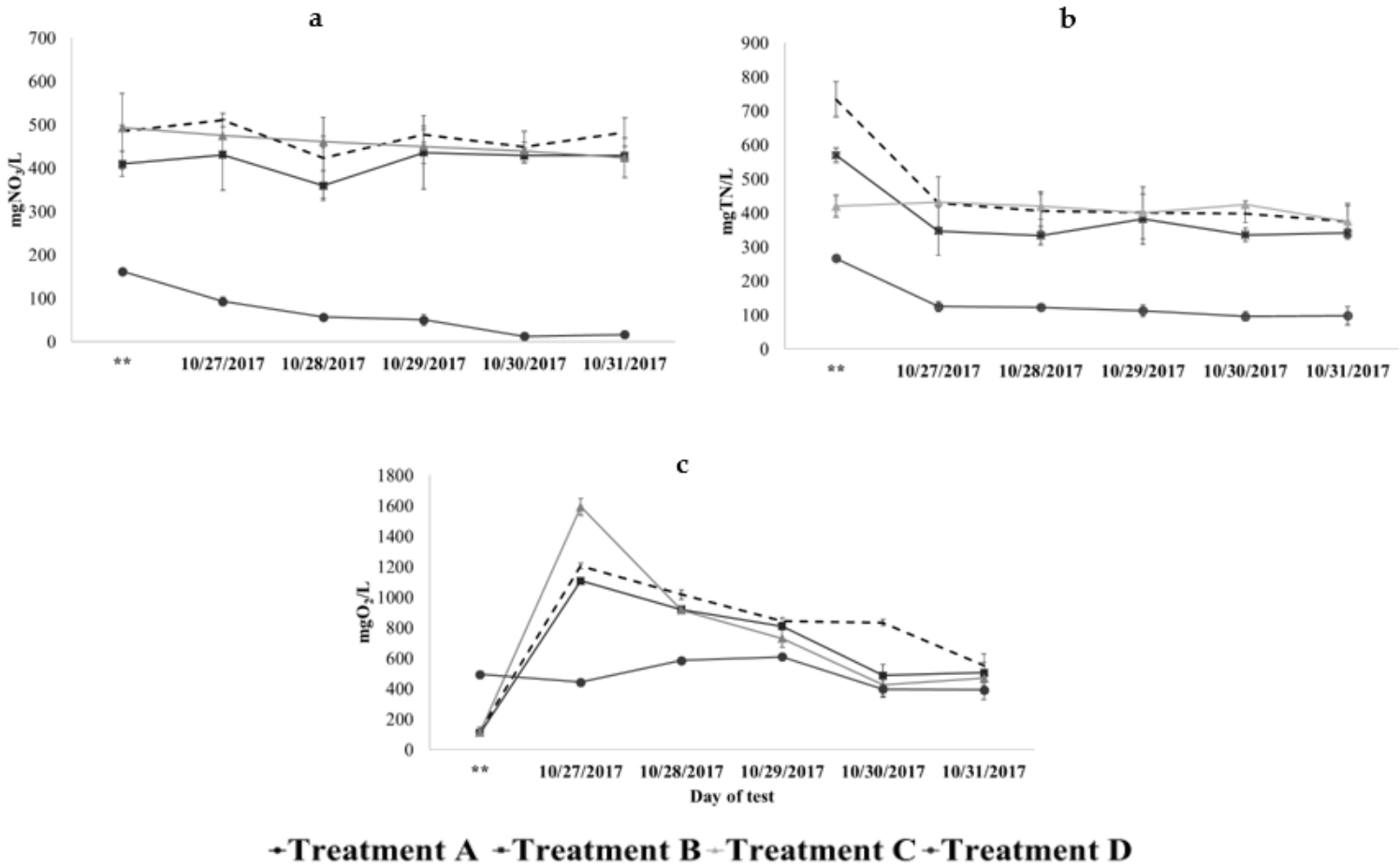

Figure 6. The mean (**a**) nitrate-nitrogen, (**b**) total nitrogen, (**c**) chemical oxygen demand concentrations mg/L after the addition of a carbon source by means of a glucose solution. (**) first sampling day controlled prior the addition of glucose solution. Treatment A (constant aeration), Treatment B (intermittent aeration 4h AA:4h no-AA), Treatment C (intermittent aeration 6h AA:4h no-AA), Treatment D (no aeration), AA = aeration.

4. Discussion

4.1. Changes in Physicochemical Profile under Different Aeration Regimes

The liquid fractions of animal manure contain high concentrations of nitrogen, phosphorus, organic compounds and micronutrients, such as copper and zinc [10,27]. The high concentrations of the two later derive from feed additives also containing iron, manganese, molybdenum and selenium [27]. The chemical and biological processes occurring within the wetland (triggered or hindered by biotic and abiotic factors) influence the concentration and/or the state of those compounds. In this study, attention was given to the effect of artificial aeration adapted to IBC systems to enhance the efficiency of COD removal from the effluent of activated sludge systems treating the liquid fraction of piggery manure.

During the presented experiment, great fluctuations in the physicochemical variables were observed. For instance, the decrease of pH values could have been influenced by the presence of organic matter, nitrification, precipitation and buffering reactions or the loss of carbon dioxide. One reason of the decrease of pH can be explained considering aerobic respiration and the corresponding CO_2 production. Nitrification is an important factor determining the pH drop. It results in the formation of carbonic acid, potentially occurring in the treatment systems A, B and C which lead to a decrease in pH. The optimal pH range for a nitrification process to occur in suspended-growth systems, such as aerated CWs or lagoons, is between 7.2–9.0 [28]. In non-wetland or conventional treatment systems, optimum nitrification has been achieved even at a pH of 6.6. By comparing the pH values with NO_3^--N concentrations after 2 weeks of the experiment (sixth sampling instance), it was seen that some of the lowest NO_3^--N values were determined when pH was approximately 6.6. The low alkalinity levels in the wastewater to resist changes in pH and neutralise acids generated by the active biomass could have also been the reason of the pH drop in treatments A, B and C. This parameter was not measured during the experiment, though it is noteworthy to consider in an optimised system for further research.

In the presence of oxygen, ions are oxidized and form precipitates, which, in consequence, decrease pH. For example, the increased concentrations of iron (Fe^{3+}) dissolved in the slurry reduce the pH if Fe^{3+} precipitates as iron phosphate ($FePO_4$(s)) or ferric hydroxide ($Fe(OH)_3$) [29]. Other possible occurring precipitates are aluminium phosphate, theoretically precipitating at pH of 6.3, or iron phosphate that ideally precipitates at a pH of 5.3 [30]. Furthermore, the precipitation processes could have been facilitated by the presence of the substrate used (Argex®). Reported studies by Meers et al. [7,10] and van Dyck, M. and van Deun, R. [31] concluded that high levels of phosphorous removal (up to 98%) were achieved at pilot scale CWs filled with Argex®.

The results also suggest that prolonged aeration periods provided to IBC A, B and C inhibited the denitrification process. Therefore, the NO_3^- concentrations were significantly reduced only in the IBC without aeration. Another reason that could have inhibited the denitrification process is the potential lack of readily degradable organic material serving as electron donors to promote the reduction of NO_3^- to molecular nitrogen (N_2) [4]. Some of the main microbial communities related to nitrogen removal are ammonia-oxidizing, denitrifying, and anammox microbial communities. Their presence, growth and reaction processes are affected by organic matter, redox condition, temperature, pH, the presence of plants and media characteristics. Most denitrifying bacteria require an anaerobic environment and readily available organic compounds for energy and as a carbon source [32]. The fractions of organic nitrogen may eventually become unavailable for microbial processes if incorporated or absorbed in the substrate. Similar results and evaluations when testing nitrogen removal by the application of intermittent aeration in microcosm wetlands were presented by Fan et al. [33] and Wu et al. [4,18]. The ammonium depletion occurred one month after the beginning of the experiment indicating that after this period, ammonia-oxidizing bacteria were readily available to promote the oxidation of ammonium to nitrite and then the oxidation from nitrite to nitrate. In general, it was evidenced that aeration altered the microbial community properties and composition. The biological activity in the aeration

systems resulted in a higher decrease of ammonium concentration due to the optimal conditions for nitrification, in contrast to the denitrification process where the nitrate depletion was limited.

The second stage of the experiment targeted a maximal decrease of the COD concentrations. The obtained removal efficiencies were comparable to the ones reported in previous studies [2,10,15,31]. Apart from the aeration provided, the use of Argex® as substrate proved to provide a better influence on COD removal than other types, such as sand or loam [10,31,34]. Nevertheless, the effect of artificial aeration could be considered as the main factor influencing recalcitrant organic matter removal, since the COD concentrations in treatment D remained high until the end of the test. Significant COD removals were obtained in the constant and intermittent aerated systems. Comparing the initial COD concentrations in Figure 4e with the initial values of Figure 6c marked as (**), it is seen that the COD average concentrations in the aerated systems decreased from approximately 1,030 mgO_2/L to levels below the discharge standard limit < 125 mgO_2/L. In contrast, S. Wu et al. [19] reported no significant COD removals.

Normally, the wetlands need a period of adaptation prior to reaching a relative steady performance. The BOD removal efficiency was comparable to the ones of COD, however, greater variation among the measurements during the experimental period were evidenced in the case of BOD. As BOD removal depends on microorganisms to degrade organic matter, it could be stated that the microbial community was better established at the latter part of this stage, when the constant decreases of the BOD concentrations were recorded in every treatment.

4.2. Hypotheses Testing Through a Mixed Model Procedure

Based on an overall judgement and estimated effects through the mixed modelling procedure, it could be deduced that treatment B and C with intermittent aeration were more efficient in terms of nutrient and organic compound removal. However, it is important to note that the optimisation of the experimental set-up and testing of different aeration rates with longer time spans are needed prior to implementation at a field-scale. Not only organic matter and nutrient removal efficiencies should be considered in this regard, but also the lowest energy consumption and maintenance costs. In this respect, the results support the fact that constant aeration implying extensive energy use would not lead to better removal efficiencies. Thus, the intermittent aerated CWs can be still considered as more sustainable, and an environmentally friendly treatment technique than other energy-intensive treatment technologies such as biological membrane reactors, activated sludge, or ammonia stripping treatment techniques, among others [35]. In addition, the implementation of aerated CWs considering similar principles as presented in this study, is economically efficient since the addition of an external carbon source, such as glucose and even plant materials, are considered low cost and non-toxic materials. To steer future research in this area, the following aspects merit attention: (i) Improving the sampling technique and handling of samples; (ii) working with continuous low flow systems to avoid concentration gradients built up in the containers and better simulations of CWs water flows; (iii) considering the wider ranges of aeration and non-aeration to allow the nitrification and denitrification process to occur in more optimal conditions. In this manner, the effects of aeration could be more effectively estimated. Guo et al. [35] averred that overly long aeration periods limit the denitrification process. However, they also claimed that further studies should be done based on high-strength wastewater types where the treatment is more complex than the existent on domestic water.

4.3. Addition of Carbon Source to Enhance The Denitrification Process

The low removal of nitrate after the addition of an external carbon source proved that aeration was the limiting factor of the denitrification process. The additional influential factors of nitrate removal are the acidic pH values (between 3.9 and 4.3) in wastewater of treatments A, B, and C fluctuated. Reported cases have shown that acidic conditions apart from influencing nitrification rates, can also inhibit denitrification [36,37]. However, it is possible that the added glucose solution was aerobically biodegraded before it could be used by denitrifying bacteria available in the containers. Furthermore,

it could be possible that the glucose solution prepared with a 3:1 COD/TN ratio was not enough to provide the necessary carbon source to promote a complete denitrification process. Guo et al. [35] in their study tested the effectiveness of recirculating the effluent, the addition of glucose, and the use of iron activated carbon stimulated with a micro electrolysis process, to decrease nitrate and ammonium concentrations. The use of an effluent with high nitrate concentrations and a controlled addition of organic content would promote the denitrification process. More effective conditions for denitrification were encountered in the non-aerated IBC. The pH values between 7.8 and 8.7, anaerobic conditions and possibly, the sufficient carbon sources, favoured the denitrification process [36,38].

4.4. Moving Towards Sustainable Concepts

The efficient decrease of the COD concentrations through the aerated wetlands could facilitate meeting the COD discharge limit of 125 mgO_2/L imposed to CWs treating animal manure. Wu et al. [39] assessing the sustainability of CWs for wastewater treatment, concluded that several tests have resulted in advanced designs, such as hybrid and/or enhanced CWs. In addition, optimal operation settings regarding the hydraulic loading rate, residence time, plant and substrate selection, number of treatment compartments, etc., have been delineated and reviewed. All of them resulted in a great increase of removal efficiencies and the sustainable application of CWs. Nonetheless, they highlighted that among the remaining gaps to meet fully optimized treatment techniques, aerated CWs should be considered for further exploration and development.

In addition, more than a post-treatment of the liquid fraction of animal manure, aerated CWs can also be optimal for the treatment of high-strength digestate, and other types of agro-industrial wastewater.

In addition, the experience and literature, which focused mostly on the treatment of urban wastewater, have shown that aerated wetlands are cheaper than non-aerated wetlands. In most practical cases, relatively low aeration periods and hydraulic retention time (HRT) of two days are general treatment considerations applied for the purification urban wastewater through CWs. Consequently, higher efficiencies and lower sludge production can be reached as compared to non-aerated systems. However, the amount of easily biodegradable organic matter in urban wastewater is higher than in the liquid fraction of pig manure. Thus, to degrade persistent organic matter (recalcitrant COD). longer retention times are required in aerated wetlands treating the liquid fraction of piggery manure.

Considering the overall manure treatment process, first, the manure is separated in solid and liquid fractions. This latter is then treated in an activated sludge system which is mostly an intensive system. Here, high aeration rates and short retention time are needed to reduce a great amount of easily biodegradable COD. In the polishing step, pig manure treatment wetlands become particularly extensive systems. The effluent of the activated sludge system containing high concentrations of non-easily biodegradable COD needs to reach concentrations below 125 mgO_2/L. To meet this criteria, longer HRT and larger treatment areas are needed in such systems. Nonetheless, to tackle land availability requirements and constraints to meet discharge limits, as discussed by Donoso et al. [6], aerated wetlands have shown to be an effective solution. Almost half of land required could treat the same amount of wastewater at higher removal efficiencies. Wastewater contains many contaminants that are in fact mixtures. Generally, water quality parameters are measured by procedures that combine individual chemical compounds into an overall or total concentration for that class of materials. Some examples of these parameters are COD, BOD or TSS. When water passes through the wetland, its composition can change as the different fractions of the mixtures can reduce at different rates. As described by Kadlec and Wallace [30], the mixture then becomes weathered. Therefore, the lower persistent COD concentrations that are aimed to be reached, the more difficult it gets to decrease their levels. However, if meeting the discharge limits is the key objective of the treatment, then one should consider that the additional costs of blowers and aeration piping would not exceed the costs of a large treatment area requirement. The use of aerated wetlands results in the reduction of the treatment area which implies that less liner, less substrate and less excavation works are needed. As a

result, the overall price of CWs' installation decreases. Compared to vertical flow wetlands, aerated wetlands are 30–50% cheaper for the same capacity. In this regard, it is suggested that aerated CW's can be combined with surface flow wetlands in a multi-stage CW, where they can replace vertical flow wetlands.

5. Conclusions

Novel technologies and strategies for wastewater treatment have been developed, tested and applied mostly throughout EU northwest countries. This is the case of enhanced CWs, which can be considered as one low-cost and efficient technology able to provide adequate removal of organics and nutrients. Considering the latter, CWs with artificial aeration were tested to intensify chemical oxygen demand (COD) removal. The results evidenced that COD removal efficiencies were higher when intermittent and continuous aeration were used in comparison than when no aeration was considered. However, the decrease of nitrogen and nitrate concentrations was not significant due to unfavourable conditions for the denitrification process (scarce anoxic conditions and available sources of organic carbon). General judgments derived from this preliminary study could presume that through the implementation of intermittent aeration in CWs, strict discharge limits imposed to CWs treating animal manure can be met. Nonetheless, further enhancement of the experimental set-up and methodologies to refine the selection of the most optimal aeration rate is needed prior to implementing this technology in the field. To conclude, this study supports the implementation of aerated CWs in areas with limited land availability as these lead to enhanced recalcitrant COD removal, reduced treatment area requirements, and lower implementation costs.

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Appendix A

Table A1. Percentage removal based on the initial and final mean values of physicochemical parameters, organic, and nitrogen compounds concentrations. Final concentrations represent the recorded values at the end of the second stage of the experiment (COD depletion test).

	pH	EC mS/cm	DO mg/L	NH_4^+-N mg/L	NO_3^--N mg/L	TN mg/L	COD mgO_2/L	BOD mgO_2/L
Treatment A								
Initial conc.	7.2	7.2	14	107	314	518	1027	27
Std Dev	±0.06	±0.06	-	±2.3	±40	±8.9	±15	±3.6
Final conc.	5.2	9.3	14	0.3	476	257	358	9.4
Std Dev	±0.06	±0.21	±0.06	0.1	±20	±47	±44	±1.2
% Removal				99	-52	50	65	66

Table A1. *Cont.*

	pH	EC mS/cm	DO mg/L	NH_4^+-N mg/L	NO_3^--N mg/L	TN mg/L	COD mgO_2/L	BOD mgO_2/L
Treatment B								
Initial conc.	7.3	7.4	14	108	344	522	1019	31
Std Dev	-	±0.06	±0.06	±0.8	±30	±25	±50	±6.1
Final conc.	5.4	9.3	13	0.3	435	298	379	8.3
Std Dev	±0.26	±0.20	±0.06	±0.2	±17	±41	±76	±2.6
% Removal				99	-27	43	63	73
Treatment C								
Initial conc.	7.3	7.5	14	110	331	525	1026	25
Std Dev	-	±0.12	±0.10	±3.7	±43	±15	±45	±9.5
Final conc.	5.4	9.3	14	0.3	463	291	434	12
Std Dev	±0.06	±0.20	-	±0.1	±22	±53	±57	±0.8
% Removal				99	-49	45	58	54
Treatment D								
Initial conc.	7.1	5.3	14	116	341	499	1057	42
Std Dev	±0.06	±0.06	±0.12	±5.4	±16	±11	±32	±2.0
Final conc.	8.1	5.2	14	75	223	222	768	34
Std Dev	±0.06	±1.0	±0.06	±2.3	±13	±65	±61	±8.5
% Removal				35	35	55	27	19

Appendix B

Table A2. Mean values of physicochemical, organic, and nitrogen compounds concentrations during the second stage of the experiment (COD depletion test).

Treatment	Sampling Day	Temp	pH	DO	EC	NH_4^+-N	NO_3^--N	TN	COD	BOD
		°C		mgO_2/L	mS/cm	mg/L	mg/L	mg/L	mg/L	mg/L
Treatment A	29/6/17	20	7.2	7.2	14	107	314	518	1027	27
	4/7/17	20	7.5	6.5	13	72	324	468	1343	51
	6/7/17	20	7.4	7.9	13	30	362	497	1157	39
	10/7/17	19	6.8	7.4	13	1.3	440	517	1023	7.1
	14/7/17	22	6.5	4.9	14	1.3	394	452	903	15
	17/7/17	23	6.9	8.6	13	1.0	371	433	832	22
	20/7/17	21	6.7	6.2	14	0.7	320	457	737	45
	24/7/17	21	6.4	6.7	14	0.5	364	475	629	4.1
	27/7/17	20	6.3	8.1	14	0.4	589	484	497	7.0
	31/7/17	19	6.0	8.9	14	0.6	539	509	520	12
	3/8/17	20	5.8	8.9	14	0.3	639	481	480	18
	7/8/17	20	5.7	11	14	0.7	379	444	438	5.4
	10/8/17	19	5.7	9.8	14	0.2	404	431	379	22
	15/8/17	21	5.5	9.2	14	0.2	580	429	499	12
	17/8/17	23	5.4	7.6	14	0.3	605	454	434	9.0
	21/8/17	21	5.3	9.5	14	0.3	558	414	377	7.8
	24/8/17	20	5.3	9.4	14	0.3	493	422	419	11
	28/8/17	22	5.2	9.3	14	0.3	476	257	358	9.4

Table A2. *Cont.*

Treatment	Sampling Day	Temp	pH	DO	EC	NH_4^+-N	NO_3^--N	TN	COD	BOD
		°C		mgO_2/L	mS/cm	mg/L	mg/L	mg/L	mg/L	mg/L
Treatment B	29/6/17	19	7.3	7.4	14	108	344	522	1019	31
	4/7/17	21	7.8	6.3	13	78	337	493	1243	58
	6/7/17	19	7.6	7.4	13	43	338	455	1090	38
	10/7/17	18	6.5	7.3	13	1.4	413	522	953	8.7
	14/7/17	23	6.5	8.1	13	0.8	383	434	886	8.0
	17/7/17	23	6.6	8.4	13	1.0	332	430	863	15
	20/7/17	21	6.6	6.7	13	0.9	261	467	756	24
	24/7/17	19	6.5	4.9	13	0.6	334	507	666	26
	27/7/17	19	6.5	7.0	13	0.6	572	489	557	5.8
	31/7/17	19	6.3	8.8	13	0.2	495	467	513	8.8
	3/8/17	20	6.0	11	13	0.3	596	471	525	16
	7/8/17	19	5.9	11	13	0.4	299	466	539	6.7
	10/8/17	19	5.8	9.7	13	0.2	393	456	433	20
	15/8/17	20	5.6	8.7	13	0.3	557	381	457	8.2
	17/8/17	23	5.5	8.0	13	0.3	532	412	434	7.5
	21/8/17	21	5.5	9.5	13	0.3	555	399	380	8.5
	24/8/17	20	5.4	9.5	13	0.3	514	444	389	9.2
	28/8/17	21	5.4	9.3	13	0.3	435	298	379	8.3
Treatment C	29/6/17	20	7.3	7.5	14	110	331	525	1026	25
	4/7/17	21	7.9	5.1	13	75	339	482	1303	70
	6/7/17	20	7.3	7.7	13	29	346	432	1097	26
	10/7/17	19	6.5	7.5	13	1.6	408	525	950	10
	14/7/17	23	6.5	8.9	13	0.8	399	452	906	11
	17/7/17	23	6.5	9.0	13	1.0	390	386	893	8.1
	20/7/17	21	6.6	7.2	14	1.0	244	420	837	12
	24/7/17	21	6.5	6.2	14	0.9	269	490	783	33
	27/7/17	20	6.5	5.8	14	0.7	513	514	683	46
	31/7/17	19	6.4	8.7	14	1.1	614	487	560	13
	3/8/17	20	6.2	12	14	0.4	625	473	553	15
	7/8/17	19	6.0	11	14	1.6	347	481	547	7.5
	10/8/17	19	5.9	9.5	14	0.2	421	460	482	24
	15/8/17	20	5.7	8.9	14	0.3	599	423	537	12
	17/8/17	23	5.7	7.4	14	0.3	340	451	443	12
	21/8/17	21	5.5	9.3	14	0.4	625	424	496	10
	24/8/17	20	5.6	9.2	14	0.3	510	497	456	12
	28/8/17	21	5.4	9.3	14	0.3	463	291	434	12
Treatment D	29/6/17	20	7.1	5.3	14	116	341	499	1056	42
	4/7/17	22	7.5	7.4	14	91	332	498	1323	65
	6/7/17	20	7.6	3.4	14	102	256	446	1153	35
	10/7/17	19	7.8	5.7	14	87	375	468	970	25
	14/7/17	22	7.8	3.2	14	83	328	387	923	20
	17/7/17	23	7.9	0.7	14	102	346	385	929	17
	20/7/17	21	8.0	1.6	14	81	214	373	860	15
	24/7/17	19	8.1	0.7	14	82	203	403	856	7.7
	27/7/17	20	8.1	1.4	14	82	318	440	803	24
	31/7/17	20	8.1	3.6	14	79	567	404	803	20
	3/8/17	20	8.1	3.9	14	71	335	397	748	26
	7/8/17	19	8.2	2.3	14	57	198	399	803	29
	10/8/17	19	8.1	2.2	14	45	199	392	800	55
	15/8/17	20	8.1	0.2	14	96	281	341	883	31
	17/8/17	24	8.0	0.4	14	87	381	311	736	29
	21/8/17	21	8.1	2.5	13	64	259	322	817	26
	24/8/17	20	8.1	4.1	14	82	248	328	849	36
	28/8/17	22	8.1	5.2	14	75	223	222	768	34

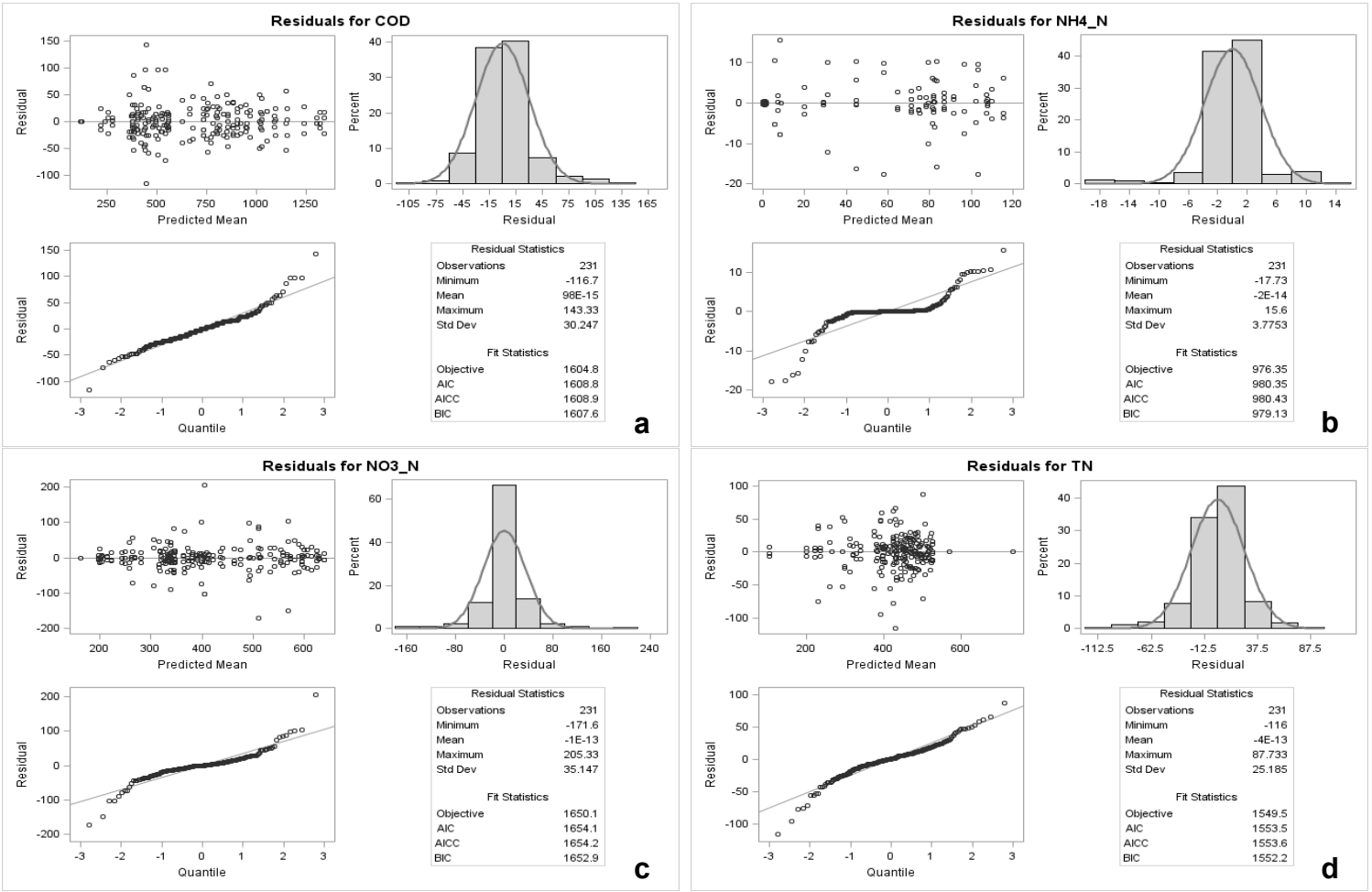

Figure A1. Diagnostic plots for the linear mixed effect model with (**a**) COD, (**b**) NH_4^+-N, (**c**) NO_3^--N and (**d**) TN as response variable and time and treatment as fixed effects. The compound symmetry covariance structure was used to account for variations in compound concentration across time for each treatment

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